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Subdivisions in the Multiple GABAergic Innervation of Granule Cells in the Dentate Gyrus of the Rat Hippocampus

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Abstract

The sources of GABAergic innervation to granule cells were studied to establish how the basic cortical circuit is implemented in the dentate gyrus. Five types of neuron having extensive local axons were recorded electrophysiologically *in vitro* and filled intracellularly with biocytin (Han *et al.*, 1993). They were processed for electron microscopy in order to reveal their synaptic organization and postsynaptic targets, and to test whether their terminals contained GABA. (1) The *hi*lar cell, with axon terminals in the *c*ommissural and *a*ssociation *p*athway termination field (HICAP cell), formed Gray's type 2 (symmetrical) synapses with large proximal dendritic shafts (n = 18), two-thirds of which could be shown to emit spines, and with small dendritic branches (n = 6). Other boutons of the HICAP neuron were found to make either Gray's type 1 (asymmetrical) synapses (n = 4) or type 2 synapses (n = 6) with dendritic spines. Using a highly sensitive silver-intensified immunogold method for the postembedding visualization of GABA immunoreactivity, both the terminals and the dendrites of the HICAP cell were found to be immunopositive, whereas its postsynaptic targets were GABA-immunonegative. The dendritic shafts of the HICAP cell received synapses from both GABA-negative and GABA-positive boutons; the dendritic spines which densely covered the main apical dendrite in the medial one-third of the molecular layer received synapses from GABA-negative boutons. (2) The *hi*lar cell, with axon terminals distributed in conjunction with the *p*erforant *p*ath termination field (HIPP cell), established type 2 synapses with distal dendritic shafts (n = 17), most of which could be shown to emit spines, small-calibre dendritic profiles (n = 2) and dendritic spines (n = 6), all showing characteristics of granule cell dendrites. The sparsely spiny dendrites of the HIPP cell were covered with many synaptic boutons on both their shafts and their spines. (3) The cell with soma in the *mo*lecular layer had an axon associated with the *p*erforant *p*ath termination field (MOPP cell). This GABA-immunoreactive cell made type 2 synapses exclusively on dendritic shafts (n = 20), 60% of which could be shown to emit spines. The smooth dendrites of the MOPP cell were also restricted to the outer two-thirds of the molecular layer, where they received both GABA-negative and GABA-positive synaptic inputs. (4) The extensive axonal arborization of the *dentate basket* cell terminated mainly on somata (n = 26) and proximal dendrites (n = 9) in the granule cell layer, and some boutons made synapses on somatic spines (n = 6); all boutons established type 2 synapses. (5) The dentate *axo-axonic* cell established type 2 synapses (n = 14) exclusively on axon initial segments of granule cells in the granule cell layer, and on initial segments of presumed mossy cells in the hilus. The results demonstrate that granule cells receive inputs from the local circuit axons of at least five distinct types of dentate neuron terminating in mutually exclusive domains of the cell's surface in four out of five cases. Four of the cell types (HICAP cell, MOPP cell, basket cell, axo-axonic cell) contain GABA, and the HIPP cell may also be inhibitory. The specific local inhibitory neurons terminating in conjunction with particular excitatory amino acid inputs to the granule cells (types 1–3) are in a position to interact selectively with the specific inputs on the same dendritic segment. This arrangement provides a possibility for the independent regulation of the gain and long-term potentiation of separate excitatory inputs, through different sets of GABAergic local circuit neurons. The pairing of excitatory and inhibitory inputs may also provide a mechanism for the downward rescaling of excitatory postsynaptic potentials, thereby extending their dynamic range.

Introduction

All cortical neurons receive GABA-mediated inhibitory input, which is essential for the normal operation of the cortical network (Krnjevic and Schwartz, 1967; for reviews see Krnjevic, 1984; Sillito, 1984). From the examination of single, identified, GABAergic, cortical cells it has become clear that they show great selectivity in the location of their synaptic boutons on the surface of the postsynaptic target cells (for review

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see Somogyi, 1989). As a result, it is highly probable that each cortical principal cell (pyramidal, spiny stellate, hippocampal granule cell) receives GABAergic synapses from different types of presynaptic local circuit neurons whose effect on the postsynaptic cell will be different, depending on the location of their synapses. Such multiple and independent innervation is relatively easy to demonstrate anatomically on the soma and proximal processes of cortical cells. For example, GABAergic input to the axon initial segment is provided by the so called axo-axonic or chandelier cell, whereas the soma and proximal dendrites are innervated by different types of basket cells (Somogyi, 1989). However, most of the GABAergic synapses in cortex are on the peripheral dendrites and dendritic spines of cortical neurons (Beaulieu and Somogyi, 1990). In the neocortex, proximal and distal dendrites coming from different layers and different types of neurons are intermingled since each area represents several superimposed versions of the basic cortical circuit. Therefore, it has not been possible to test whether GABAergic synapses on the distal dendrites of the same cell come from a different source from those on the more proximal dendrites.

This difficulty can be overcome in the archicortical area, the hippocampal formation, where the cell bodies of principal cells are lined up in close proximity, and as a result equivalent parts of the dendritic segments are also at the same location, arranged in a laminar fashion. This structural principle reveals laminar segregation of inputs to the principal cells and has made possible detailed anatomical and physiological examination of synaptic organization in the hippocampus (for reviews see Andersen, 1975; Buzsaki, 1984; Schwartzkroin and Mueller, 1987; Lopes da Silva *et al.*, 1990) not yet possible in the neocortex. The arrangement and role of the main excitatory pathways is reasonably well known (Ramon y Cajal, 1893; Lorente de No, 1934; and see references above), but the organization of the accompanying inhibitory connections is less clear and is based on inferential evidence. In the present study we exploited the strict laminar organization of the dentate gyrus of the hippocampal formation to examine whether different parts of one of the principal cells, the granule cell, receive independent GABAergic inputs.

The dentate gyrus is the gate to the hippocampus from the entorhinal cortex through which afferents activate the trisynaptic circuit of the hippocampal formation. The principal granule cells projecting to the CA3 area are innervated by the entorhinal excitatory pathway terminating in the outer two-thirds of the molecular layer, and by commissural and associational excitatory pathways terminating more proximal to the somata in the inner third of the molecular layer (Steward, 1976). In addition they also receive input from several types of dentate cells having local circuit axons. Many of the latter neurons are thought to use GABA as transmitter, since a high percentage of hilar cells and up to 2% of neurons in the granule cell layer are immunopositive for glutamate decarboxylase (GAD) and GABA (Ribak *et al.*, 1978; Woodson *et al.*, 1989). Immunoreactivity for GABA has also been demonstrated in identified dentate axo-axonic cells terminating on granule cell axon initial segments (Soriano and Frotscher, 1989). The somata of granule cells also receive GAD-immunoreactive synaptic boutons (Ribak *et al.*, 1978; Kosaka *et al.*, 1984), which are thought to originate from the so-called basket cells (Ramon y Cajal, 1893; Lorente de No, 1934; Seress and Ribak, 1990). There is much less information about the distribution of inhibitory synapses on different parts of the granule cell dendrites.

Granule cells respond to exogenous GABA by depolarization or hyperpolarization depending on the membrane potential and the site of application (Misgeld *et al.*, 1986; Blaxter and Carlen, 1988). The activation of entorhinal or commissural afferents produces both excitation and GABA-mediated feed-forward inhibition (Buzsaki and Eidelberg, 1981; for review see Buzsaki, 1984). Using intracellular recording *in vitro*, inhibitory postsynaptic potentials (IPSPs) were evoked through both $GABA_A$ and $GABA_B$ receptors in addition to excitatory postsynaptic potentials (EPSPs) (Rausche *et al.*, 1989; Muller and Misgeld, 1990). Inhibitory postsynaptic currents mediated by GABA and evoked by the activation of local dentate cells have also been demonstrated (Edwards *et al.*, 1990). However, the location of the synapses producing the GABA-mediated inhibition has not been identified in any of the studies. The location may be important because it has emerged that long-term potentiation (LTP) (Bliss and Lomo, 1973), a prominent phenomenon in the dentate gyrus, depends on the level and mode of GABA-mediated inhibition (Douglas *et al.*, 1982; Collingridge *et al.*, 1988; Davies *et al.*, 1990, 1991). Accordingly, in circuits having multiple segregated excitatory inputs, GABAergic cells may play selective or generalized roles depending on their input/output relationships to granule cells.

The visualization of hilar cells having extensive local circuit axons with great spatial selectivity, demonstrated in the accompanying paper (Han *et al.*, 1993), provided an opportunity to examine the source of GABAergic innervation to granule cells. The results show that granule cells receive GABAergic innervation from different types of neurons which subdivide the surface of granule cells in a spatially selective manner.

Material and methods

Tissue preparation

Young adult, female Wistar rats were used. The technical details of the preparation of hippocampal slices, electrophysiological recording, filling of neurons and the procedure for the visualization of biocytin-filled cells are described in the accompanying paper (Han *et al.*, 1993). Briefly, the animals were intracardially perfused with artificial cerebrospinal fluid under ether and ketamine anaesthesia. Horizontal hippocampal slices were cut and recorded in an interface-type recording chamber. After brief physiological characterization, 2% biocytin dissolved in 1.5 M KCH_3SO_4 was iontophoretically delivered into the identified neurons for 5–10 min. Thirty to sixty minutes were allowed for the transport of the marker, then the slices were fixed overnight in a fixative containing 2.5% paraformaldehyde, 1.25% glutaraldehyde and 15% saturated picric acid in 0.1 M phosphate buffer. Slices were freeze-thawed in liquid nitrogen, embedded in gelatine, and 60 μm thick sections were cut and processed for the visualization of the biocytin-filled cells with avidin–biotinylated peroxidase complex (Vector Laboratories). The peroxidase was visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) as chromogen. After 1 h postfixation in 1% osmium tetroxide the sections were block-stained with 1% aqueous uranyl acetate, dehydrated and flat-embedded on glass slides in Durcupan ACM epoxy resin (Fluka).

Electron microscopy and postembedding immunogold reaction for GABA

Axon-rich areas were selected under a light microscope and re-embedded for ultrathin sectioning. Serial sections were cut and mounted on single-slot Formvar-coated copper grids, stained with lead citrate and used for studying the morphology and synaptic connections of the identified neurons. Serial sections mounted on nickel grids were used to reveal GABA immunoreactivity with the postembedding immunogold technique (Somogyi and Hodgson, 1985), using a recently introduced gold-labelled antibody. The reaction was carried out on droplets in humidified Petri dishes. The resin was etched with 1% periodic acid for 10 min, and osmium was removed from the sections with 4% sodium metaperiodate treatment for 20 min. The primary antiserum to GABA (code no. 9;

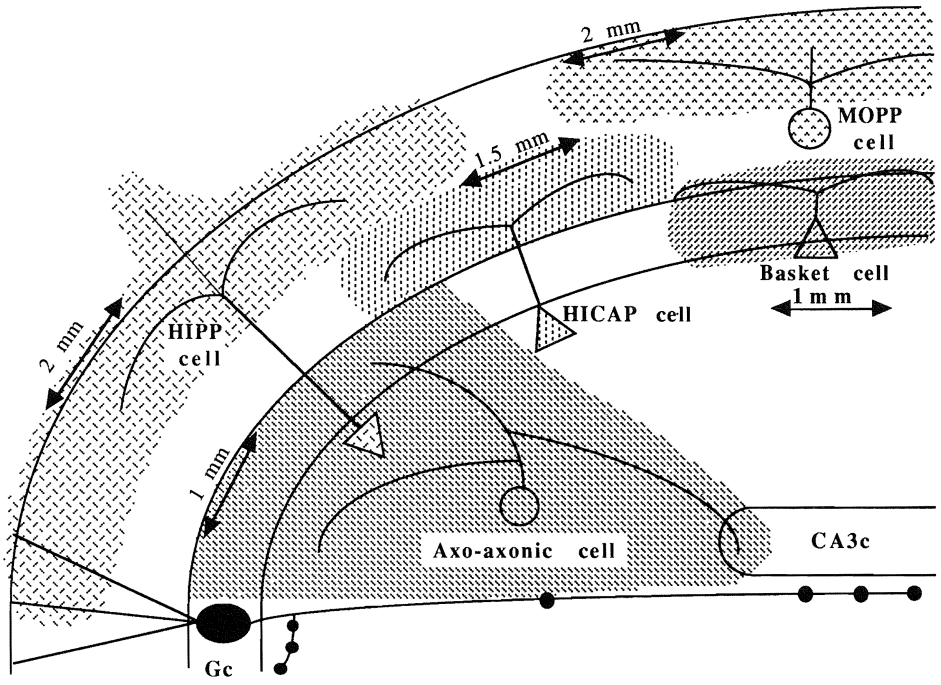


FIG. 1. Schematic drawing representing the distribution of the axon arborizations of five types of local circuit GABAergic neuron in the dentate gyrus of the rat. 1. Hilar cell with an axon associated with the commissural association pathway (HICAP cell). 2. Hilar cell with an axon associated with the perforant pathway (HIPP cell). 3. Neuron in the molecular layer having an axon associated with the perforant pathway (MOPP cell). 4. Basket cell with soma at the border of the granule cell layer and its axon covering the granule cell layer, the border region of the molecular layer and sending axon collaterals also to the hilus. 5. Axo-axonic cell having an axon covering the granule cell layer and most of the hilus. For detailed reconstructions see accompanying paper by Han *et al.* (1993). The values over the bars indicate the extent of the axonal field detected in 400-μm thick slices.

Hodgson *et al.*, 1985) was used overnight at a dilution of 1:1000 at 4°C and the secondary antibody, goat-anti rabbit immunoglobulin G (IgG) covalently coupled to 1-nm gold particles (Nanoprobes, Stony Brook, USA), for 1 h at a dilution of 1:100 at room temperature. In other experiments anti-rabbit IgG conjugated to 15-nm gold particles was also tried; however, this reagent generally resulted in a low signal-to-noise ratio. Tris-buffered saline containing 1% normal goat serum was used for the washing steps, and for the dilution of the primary antiserum. The secondary antibody was dissolved in 50 mM Tris buffer (pH 7.4) containing 1% bovine serum albumin and 0.5% Tween 20. After thorough washing and postfixation with 1% glutaraldehyde in phosphate-buffered saline, sections were placed on distilled water droplets. The visualization of the bound 1-nm gold-conjugated antibody was carried out by silver intensification with the HQ SILVER kit (Nanoprobes) for 4 min. Following the immunostaining and silver intensification procedures the sections were contrasted with a saturated aqueous solution of uranyl acetate for 30 min and with lead citrate for 2 min.

Evaluation of the postembedding immunoreaction

Immunoreactions using secondary antibodies conjugated to 15-nm gold particles resulted in a low signal-to-noise ratio, although the same reagent gave good results in perfusion-fixed, simultaneously processed material. Thus, the likely reason for the poor reaction was the loss of GABA from slices kept in the recording chamber for several hours (Mihaly *et al.*, 1991) and the slow penetration of fixative into the slice. These factors caused a higher background level and at the same time the loss of GABA from GABAergic cells reduced the signal in the terminals. In addition the persisting DAB–OsO_4 precipitate filled the boutons of the labelled cells and masked GABA antigenic sites, leading to further reduction in signal.

In order to overcome the above technical difficulties, the duration of sodium periodate treatment and incubation with the primary serum were increased, and a secondary antibody, covalently bound to 1-nm gold particles and visualized by silver intensification, was used as described above. This more sensitive procedure improved the immunocytochemical signal and made possible the detection of GABA both in biocytin-filled and non-filled profiles of some, but not all, of the hippocampal slices. The selective reduction of GABA content in some slices, or in some cell types, might lead to false negative results even with this sensitive method. Thus, only positive results can be interpreted with certainty.

Immunocytochemical control reactions

To ascertain method-specificity in the reaction, incubation was also carried out with primary antiserum preadsorbed to GABA conjugated to polyacrylamide beads as described earlier (Hodgson *et al.*, 1985). Selective deposition of metal particles was not observed under this condition, showing that the reaction observed with the full anti-GABA serum was due to specific antibodies. This control reaction also shows that peroxidase reaction end-product on its own does not produce the reduction of silver under our conditions. Silver deposition was not observed in the HIPP cell, or in the dendrites of a biocytin-filled granule cell, further demonstrating that the selective reactions are due to the gold-labelled secondary antibodies. The primary antiserum to GABA has been characterized for antibody specificity, and has been found to show not more than 10% cross-reactivity with γ-amino-β-hydroxybutyrate, β-alanine, δ-aminovalerate and ϵ-amino-caproate (Hodgson *et al.*, 1985). None of these substances are known to be concentrated in neurons; therefore the selective localization of immunoreactivity most likely reflects the distribution of GABA.

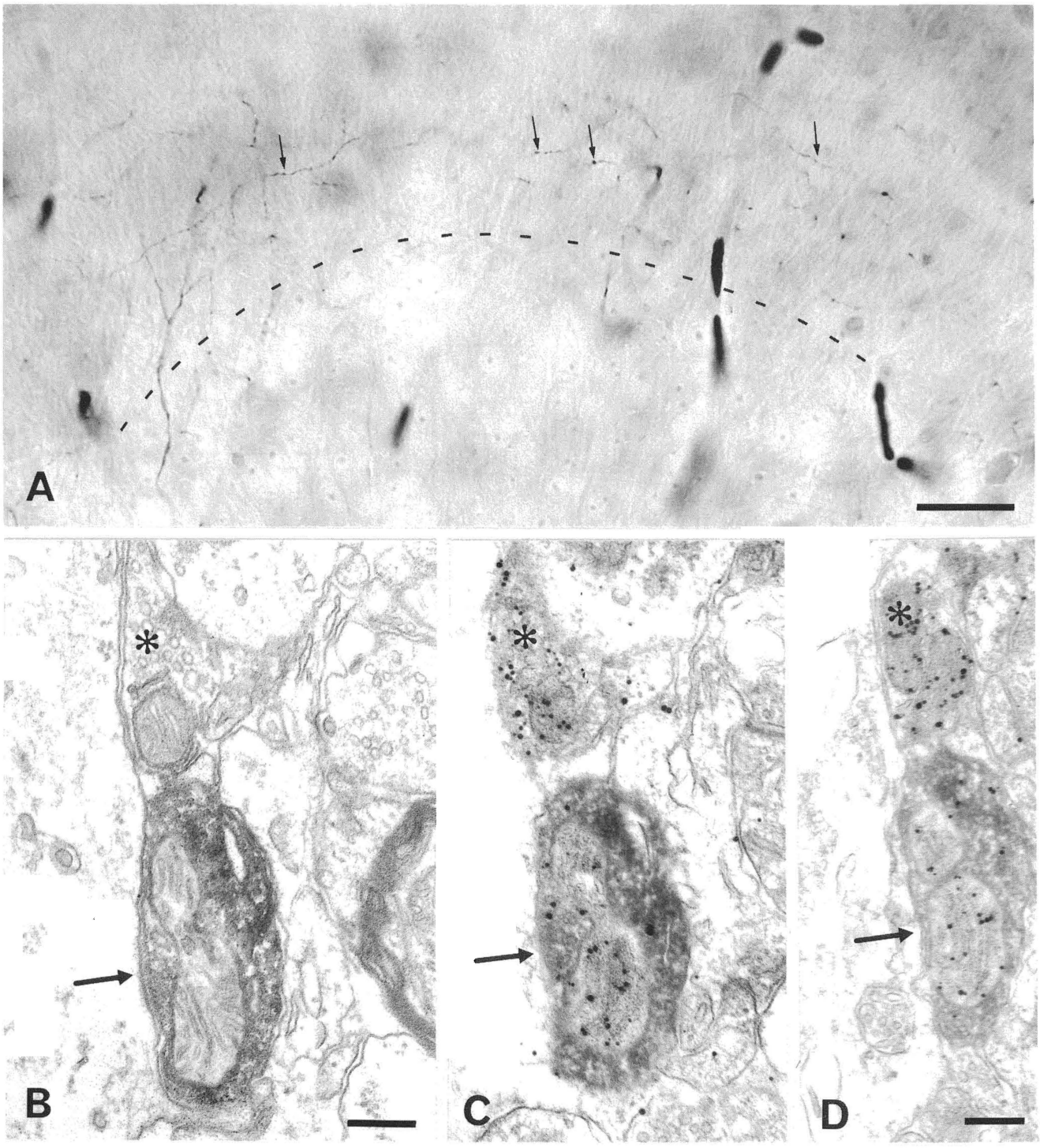


FIG. 2. Light (A) and electron micrographs (B–D) of the HICAP cell. (A) Characteristic distribution of HICAP cell axon, showing varicosities (arrows) in the inner molecular layer. The broken line follows the border of the granule cell layer with the molecular layer. (B–D) Type 2 synaptic contact (arrow) between a biocytin-filled bouton and a dendritic shaft. The alternate serial sections in C and D were immunoreacted for GABA. Both the biocytin-filled bouton and another non-filled bouton (asterisk) containing large ovoid vesicles are GABA-immunopositive, as shown by the high density of silver-intensified gold particles over them. The postsynaptic dendrite is GABA-immunonegative. Scales: A, 50 μm; B–D, 0.2 μm; B and C are at the same magnification.

Results

Location and termination field of the identified neurons

Three of the five types of intracellularly filled neurons were named on the basis of the location of their somata and the overlap between their axonal projection and the termination field of other hippocampal afferents, whereas two of them were similar to previously described basket and axo-axonic cell types. The detailed documentation of their axonal and dendritic arborizations can be found in the accompanying paper (Han

et al., 1993). Here we give a brief overview as illustrated schematically in Figure 1.

The HICAP cell (*hi*lar cell with an axon in the *c*ommissural and *a*ssociational *p*athway termination field) was a multipolar neuron with soma beneath the granule cell layer and axonal field in the inner one-third of the molecular layer (Figs 2–4). The majority of the dendrites, branching in all subfields of the dentate gyrus, were smooth or beaded, but the main dendrite gave rise to spines on its segment in the central one-third of the molecular layer. The HIPP cell (*hi*lar cell having an axon associated with the *p*erforant *p*ath projection) was a multipolar neuron having a fusiform cell body situated beneath the granule cell layer, and an axon arborizing in the outer two-thirds of the molecular layer (Fig. 5). Its sparsely spiny dendrites were branching exclusively in the hilus, mainly in the polymorph layer. The MOPP cell (*mo*lecular layer neuron having an axon associated with *p*erforant *p*ath input) was also multipolar; the soma was in the inner one-third of the molecular layer and both the dendrites and axon arborized in the outer two-thirds of the molecular layer (Figs 6 and 7). The *dentate basket cell* soma was at the border of the granule cell and polymorph layers; the dendrites of this cell were not recovered. The main axon travelled from the injection site, crossing the granule cell layer to the molecular layer, where it emitted thick tangential collaterals and sent its varicose terminal branches back to the granule cells (Figs 8 and 9). The fifth neuron type was a *dentate axo-axonic cell*, its soma located in the hilus and a very extensive axon collateral system carrying its chandelier-like rows of large boutons distributed in the granule cell layer and the hilus (Fig. 10). The nearly parallel varicose axon branches ran at right-angles to the granule cell layer, following the direction of the axon initial segments of granule cells. Very few of the dendrites were recovered in the dentate molecular layer.

Synapses, postsynaptic targets and synaptic input of local circuit neurons

HICAP cell

The main axon branches of the HICAP cell travelled through the granule cell layer and gave rise to numerous thin and frequently branching collaterals in the inner molecular layer over the granule cell bodies (Fig. 2A). The biocytin-filled axons were easily recognisable by the dense osmium-stained precipitate (Figs 2 and 3). The axon provided a high density of synaptic boutons in the inner one-third of the molecular layer. The shape and size (0.4–0.9 μm) of the presynaptic varicosities were varied, but they always contained synaptic vesicles and very often mitochondria. Altogether 34 synaptic boutons were examined in serial sections (Table 1), and 18 of them made synapses with large proximal dendritic shafts containing mitochondria and microtubules (Fig. 2B). Spines could be identified originating from 12 of the 18 postsynaptic dendrites. A further six boutons of the HICAP cell made synapses with small-calibre dendrites without mitochondria, but containing microtubules. Each of the above 24 boutons gave type 2 symmetrical synapses (Gray, 1959). A further ten biocytin-filled synaptic boutons were found to form synapses with dendritic spines (Fig. 3B) or, in one case, with a spine neck (Fig. 3A). Six of these terminals established type 2 synapses (Fig. 3A, B), whereas the remaining four established type 1 asymmetrical synapses (Fig. 3F, G). It was unusual that the same individual cell formed two different types of synapses, so the axons giving rise to the asymmetrical synapses were followed in serial sections in order to establish whether the same axon made both types of synapses. In this way, the axon collateral providing a type 1 synapse and shown in Figure 3F was found to establish a type 2 synapse at a distance of ~20 μm from the other synapse. Four of the spines receiving a synapse from the HICAP cell also received another synapse from an unlabelled bouton forming a type 1 junction (Fig. 3A–C).

The dendrites of the HICAP cell were strongly filled with precipitate, so the only recognizable structures were mitochondria. Many non-filled boutons formed synapses on both the smooth and the spiny part of the dendrites (Fig. 4) and some of the boutons were GABA-immunoreactive on both the smooth (Fig. 4B, C) and spiny segments (Fig. 4D). The large irregularly shaped dendritic spines were covered with synapses established by small terminals, which were GABA-immunonegative and contained round synaptic vesicles (Fig. 4E).

HIPP cell

The axon branches ascended straight up to the outer molecular layer where they arborized densely through frequent Y-shaped bifurcations (Fig. 5A). The axon collaterals carried numerous boutons 0.2–1 μm in diameter (Fig. 5B). Synapses were also established by thin non-varicose segments of axons densely packed with synaptic vesicles (Fig. 5C, D). All the examined boutons of the HIPP cell formed type 2 synapses. The postsynaptic targets (Table 1) were dendritic shafts (n = 17), of which 15 could be shown to emit spines (Fig. 5C), small dendritic branches (n = 2) and dendritic spines (n = 6, Fig. 5D). Four of the spines receiving a synapse from the HIPP cell also received another synapse from an unlabelled bouton forming a type 1 junction (Fig. 5D); the other two spines were not followed through in serial sections to establish whether they received other synapses. The dendrites of the HIPP cell in the hilus gave rise to large irregularly shaped spines densely covered with synaptic terminals (Fig. 5E).

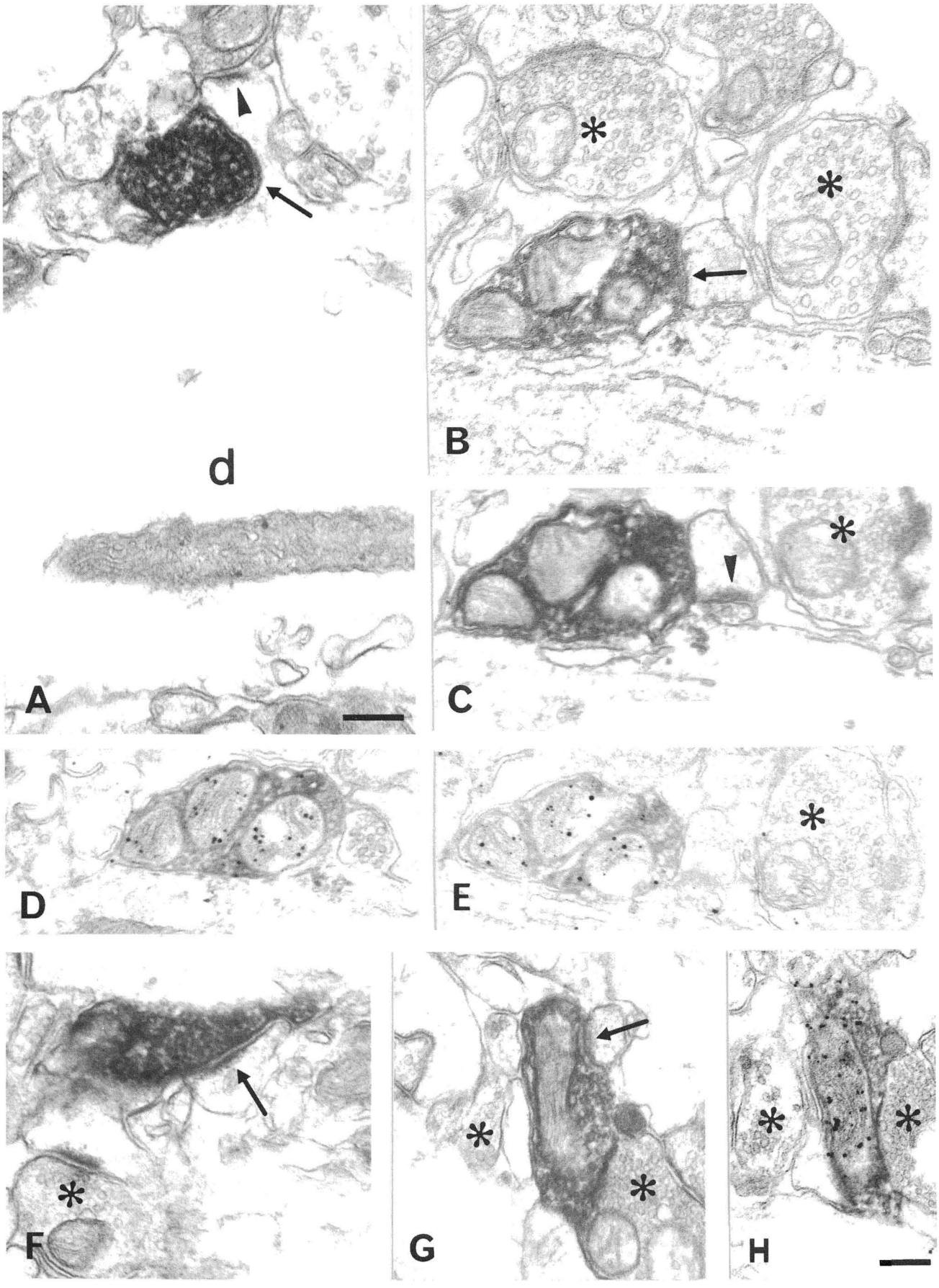
MOPP cell

The axon collaterals of the MOPP cell, distributed in an umbrella-like fashion, occupied the outer molecular layer. The individual branches ran for hundreds of micrometers without branching and they were less densely varicose than the axons of the first two cell types (Fig. 6A). Both the varicosities (Fig. 6B) and the thin non-varicose parts of the MOPP cell axon (Fig. 6C, D), containing synaptic vesicles, formed synaptic contacts. All 20 examined synapses of the MOPP cell were of type 2 in character, and all postsynaptic targets were dendritic shafts (Table 1). Spines were found originating from 12 postsynaptic dendrites, whereas no spines were recognizable on the remaining eight dendrites. However, taking into account the suboptimal preservation of the tissue, the presence of spines on these dendrites cannot be excluded. The dendrites of the MOPP cell were smooth or beaded (Fig. 6A), and except for the mitochondria they were strongly filled with the dense precipitate (Fig. 7C). They received synapses from many non-filled boutons of various sizes, some of which were immunopositive for GABA (Fig. 7D).

Dentate basket cell

A thick main axon emerged from the injection site, where the remnants of a soma were found under the electron microscope. The main axon travelled straight to the inner molecular layer, where it gave rise to secondary collaterals in a T-shaped manner (Fig. 8A). Thin varicose branches originated from these secondary axons, turned back to the granule cell layer and formed a dense plexus among the granule cells (Fig. 8A).

The area chosen for studying the synapses included the thick unmyelinated main axon of the basket cell (Fig. 8B), which contained a high density of mitochondria, and did not receive synaptic input. The secondary branches were also unmyelinated. The thin collaterals had frequent synaptic boutons, which often aligned themselves with the somata of granule cells (Figs 8C and 9B). Usually each bouton made one type 2 synaptic junction. Altogether 41 basket cell synapses were identified, of which 26 were on somata showing the morphological characteristics of granule cells (Fig. 9A–C). Six synapses were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established with somatic spines (Fig. 8C), and nine biocytin-filled basket boutons terminated on proximal dendrites at the top of the granule cell layer. Spines were identifiable on six postsynaptic dendrites, whereas three did not emit spines in the plane of the sections. Although the granule cells were not completely sectioned, the majority contacted by the filled basket axon seemed to receive only one synapse from the identified basket cell. One of the postsynaptic granule cells received synapses from two filled boutons (Fig. 9A–C), and another one received two synapses from the same varicose axon. However, within the termination field of the identified basket cell many granule cells did not seem to receive synapses from biocytin-filled boutons. Non-filled terminals, establishing similar junctions to the identified basket cell terminals, were also found in synaptic contact with the same granule cells that received the biocytin-filled boutons (Fig. 9A, B).

Dentate chandelier cell

This cell type is characterized by its chandelier-like rows of boutons following the direction of the axon initial segments of granule cells (Fig. 10A), or of other neurons in the hilar region. The main axon originating in the hilus from a disintegrated soma was myelinated, as were the main secondary and tertiary collaterals. The axo-axonic cell had the largest boutons (0.5–1.5 μm) among the five studied cell types (Fig. 10). All 14 biocytin-filled boutons examined were found in type 2 synaptic contact with axon initial segments (Fig. 10B, C). Twelve of the boutons were in the granule cell layer and two in the hilus. The axon initial segments, identified by the electron-dense membrane undercoating and occasional microtubule fascicles, received synapses from many unlabelled boutons giving type 2 symmetric synapses (Fig. 10B, C) in addition to the biocytin-filled boutons. For example, on a small portion of one of the longitudinally cut axon initial segments (Fig. 10B) three filled and seven non-filled synaptic boutons, probably provided by other chandelier cells, could be counted.

GABA immunoreactivity of the identified neurons

Axo-axonic cells are likely to use GABA as a transmitter since they were shown to be immunoreactive for GABA in the CA1 region and in the dentate gyrus (Somogyi *et al.*, 1985; Soriano and Frotscher, 1989). Terminals making type 2 synapses on somata of granule cells and probably originating from basket cells are immunoreactive for GAD (Ribak *et al.*, 1978) and probably also use GABA as a transmitter. The possible transmitter of the other three cell types studied here is not known, but since they make type 2 synapses, many of which are given by boutons immunoreactive for GAD or GABA in the dentate gyrus, GABA is a likely transmitter candidate in these cells. Therefore we applied a sensitive postembedding immunogold method to test if any of the boutons formed by the biocytin-filled HICAP, HIPP and MOPP cells were immunoreactive for GABA.

The HICAP cell was GABA-immunoreactive (Figs 2 and 3). From the 37 synaptic boutons described above, 12 were found in sections immunoreacted for GABA and were immunopositive, including the ones making type 1 asymmetrical synapses (Fig. 3H).The postsynaptic targets were five dendritic spines, three dendritic shafts and three small dendritic profiles without mitochondria, and they were all GABA-immunonegative. The mitochondria in the dendrites of the HICAP cell were free of peroxidase reaction end-product, and they were also immunopositive for GABA (Fig. 4B, C, E).

The GABA reaction was ambiguous in the case of the HIPP cell. The reaction in each trial resulted in a high background of gold/silver labelling and the terminals were also very densely filled with peroxidase precipitate, which may have contributed to the lack of specific immunoreactivity signal. Nevertheless, occasional non-filled synaptic boutons were immunopositive in the neuropil.

The slice containing the MOPP cell reacted well for GABA, and processes of the cell were found to be immunopositive (Fig. 7). The axons and the six tested vesicle-containing boutons were strongly labelled with silver-intensified gold particles (Fig. 7A, B). Unfortunately, no synaptic junctions were found in the material reacted for GABA, thus the GABA content of the postsynaptic targets could not be assessed.

Discussion

The spatially selective local axonal arborizations originating from dentate neurons, described by Han *et al.* (1993), have all been shown to form type 2 or symmetrical synapses (Gray, 1959), but they have different postsynaptic target selectivity. In addition, the HICAP cell also forms type 1 synapses on dendritic spines. The terminals of the HICAP and the MOPP cells have been shown to contain GABA, thus demonstrating that dentate granule cells receive GABAergic inputs on their dendrites independent of the GABAergic neurons supplying their soma and proximal processes.

Postsynaptic target selectivity of local circuit axons

The five local circuit cells examined here in the dentate gyrus are homologous in their postsynaptic target selectivity to cortical local circuit neurons (Somogyi, 1989) and, as expected, demonstrate similarity in the organization of the basic cortical circuit throughout the cortex.

Chandelier or *axo-axonic cells* have been described in the dentate gyrus (Kosaka, 1983; Soriano and Frotscher, 1989; Soriano *et al.*, 1990) and, confirming previous studies, have been found to make synapses exclusively with axon initial segments of granule cells and probably of mossy cells in the hilus. The latter assumption is based on the homologous morphology and synaptic organization of mossy cells and pyramidal cells (Frotscher *et al.*, 1991).

Basket cells are generally accepted as the principal inhibitory neurons in the dentate gyrus (for review see Buzsaki, 1984; Lubbers and Frotscher, 1987; Lopes da Silva *et al.*, 1990) and one or more of the following assumed or partially proven characteristics have been thought to identify them in previous studies: (i) somata in or near the granule cell layer; (ii) axon arborization in the granule cell layer; (iii) synapses provided to the somata of granule cells; (iv) they contain markers for GABAergic neurotransmission. Previous identifications based only on the first two criteria can no longer be used alone for identifying basket cells. For example, the HICAP cell also has its pyramid-shaped soma near the granule cell layer, and the chandelier cells provide at least as many terminals, if not more, in the granule cell layer, as do genuine basket cells, but neither of the first two types of neuron make synapses on the somata. The location of basket cell synapses, proven to originate

FIG. 3. Spines as synaptic targets of the HICAP cell. (A) Biocytin-filled bouton giving a type 2 symmetrical synapse (arrow) to the neck of a spine originating from a large-diameter dendrite (d). The same spine also receives a type 1 asymmetrical synapse (arrowhead). (B–E) Serial sections of another bouton making a synaptic contact (arrow in B) with a dendritic spine receiving also a type 1 synapse (arrowhead in C). Asterisks mark non-filled boutons giving asymmetrical synapses on dendritic spines. Silver-intensified immunogold reaction for GABA (D, E) shows that the bouton of the HICAP cell is GABA-immunopositive. (F, G) Type 1 synapses (arrows) made by the boutons of the HICAP cell on dendritic spines. (H) Serial section to G, showing GABA immunoreactivity of the filled bouton. Asterisks mark non-filled boutons. Scales: 0.2 μm; A–F and G, H are at the same magnification.

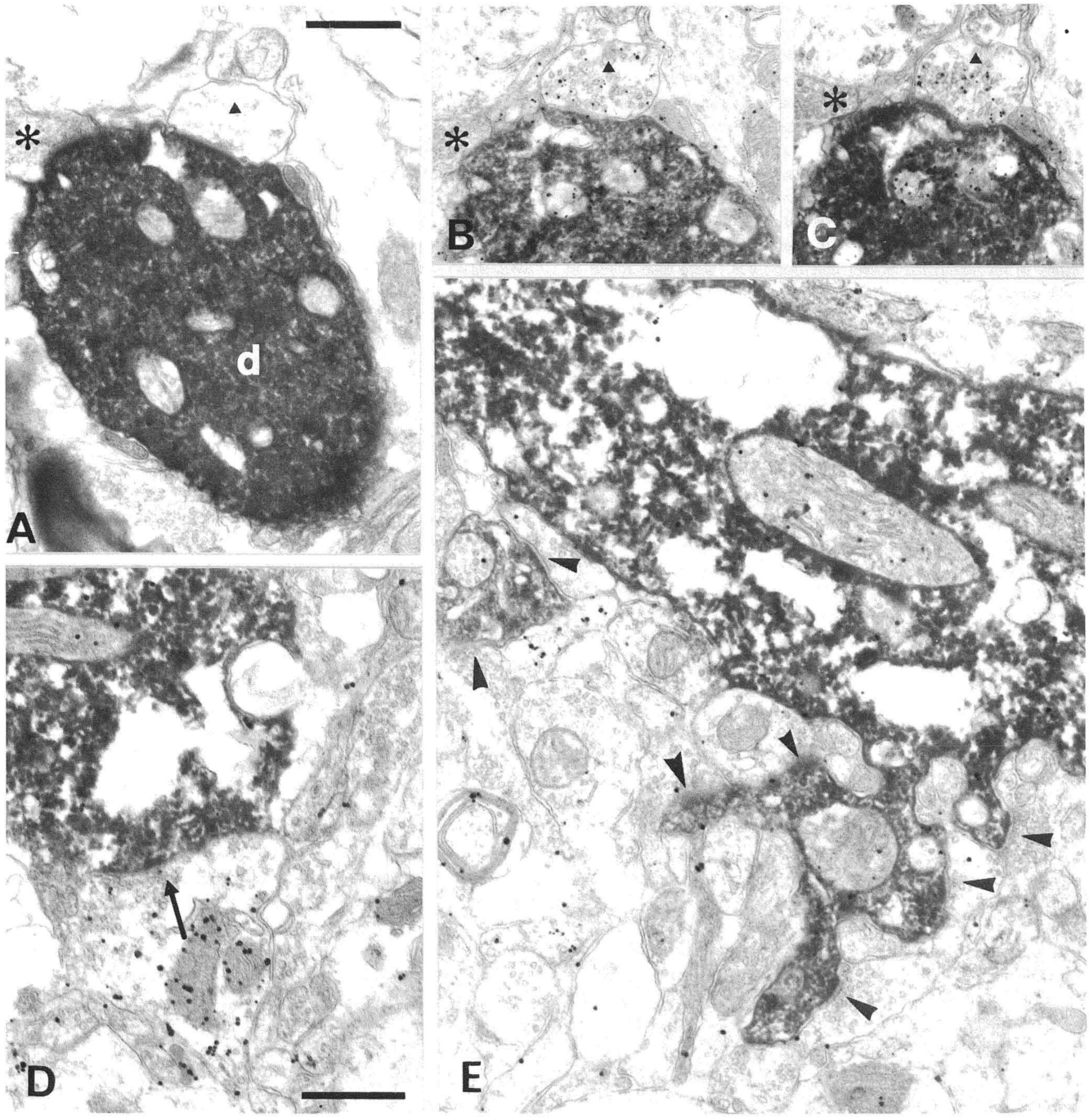

FIG. 4. Synaptic input to the HICAP cell. Sections shown in B – E were immunoreacted for GABA by the silver-intensified postembedding gold reaction. (A – C) Serial sections of the smooth part of the main dendrite (d) in the inner molecular layer, receiving synapses from a GABA-positive (triangle) and a GABA-negative (asterisk) axon terminal, as shown in B and C. (D, E) The spiny part of the same dendrite in the outer molecular layer receives a GABA-positive synaptic bouton (arrow in D) on the dendritic shaft, and numerous GABA-negative synaptic boutons (arrowheads on E) on the dendritic spines. Scales: A – C, same magnification, 0.5 μm; D and E, same magnification, 0.5 μm.

from identified local cells, has only been examined for two cells in developing dentate gyrus (Seress and Ribak, 1990) and the cells examined were found to make synapses with somata as well as with dendrites (60 and 36% of targets). This has been confirmed by our results from adult material, but showing a higher proportion of somatic synaptic targets (78%). Therefore, it seems appropriate to use as a criterion the quantitative distribution of postsynaptic targets for defining basket cells in a strict sense. Finally, to call all GABA- or GAD-immunopositive neurons 'basket cells' without identification of their axons is clearly no longer justified, as several cell types avoiding the somatic region of granule cells with their terminals are also immunoreactive for GABA.

To make a distinction between different types of local circuit neurons is not proposed for semantic reasons. We have shown great specificity in synaptic target selectivity of local cells predicting differences in action.

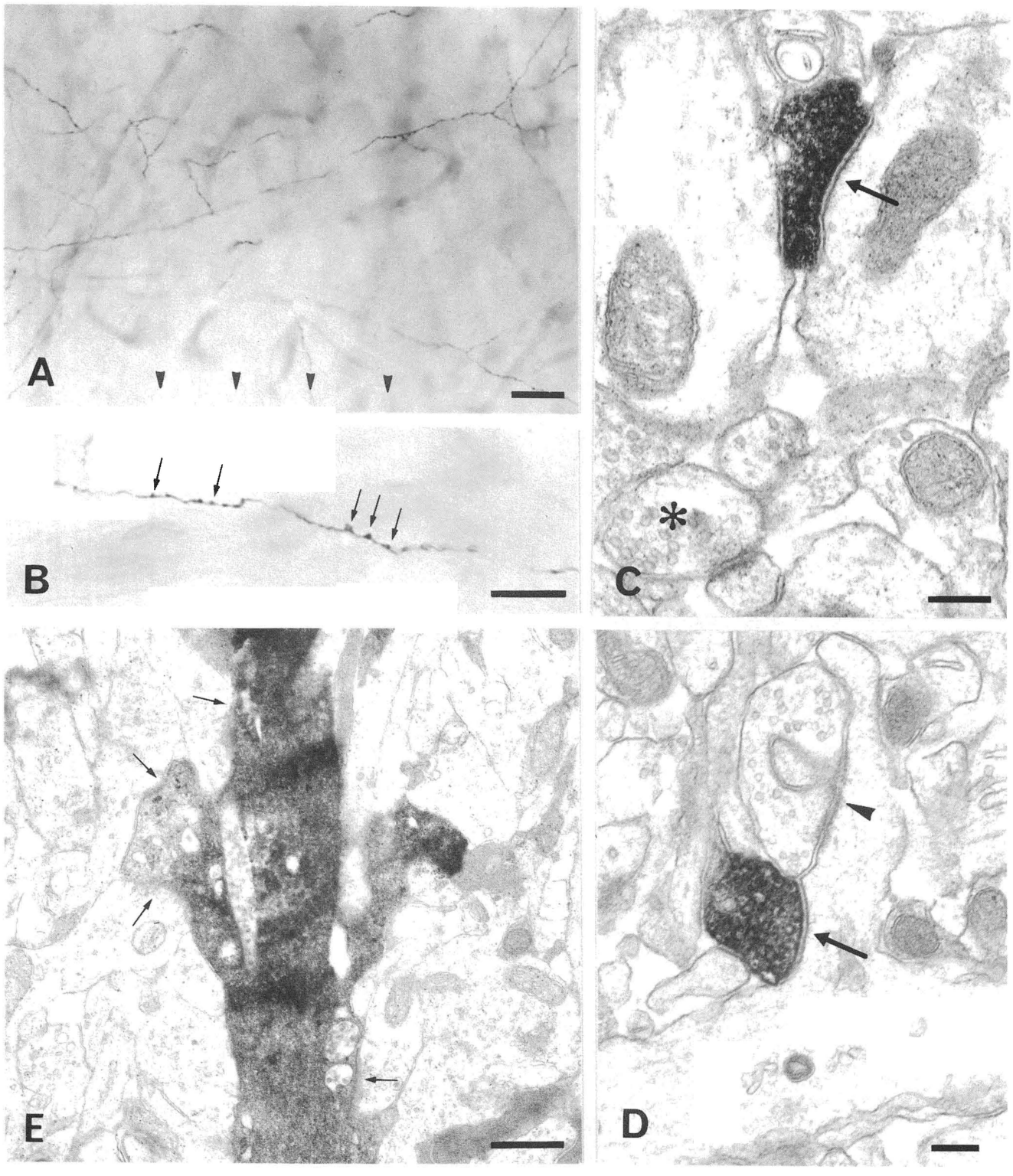

FIG. 5. Light (A, B) and electron (C – E) micrographs of the HIPP cell. (A, B) Characteristic density of HIPP cell axon, showing varicosities (arrows) in the outer molecular layer. Arrowheads point towards the granule cell layer. (C) Type 2 or symmetrical synapse (arrow) between a biocytin-filled axonal varicosity and a dendritic shaft. The asterisk marks a non-filled bouton giving an asymmetrical synapse on a spine. (D) Type 2 synapse (arrow) between a biocytin-filled bouton and a long dendritic spine, which also receives another synapse (arrowhead) from a non-filled bouton. (E) Detail of a HIPP-cell dendrite in the hilus, receiving numerous synapses (arrows) both on its shaft and spines. Scales: A, 20 μm; B, 10 μm; C and D, 0.2 μm; E, 0.5 μm.

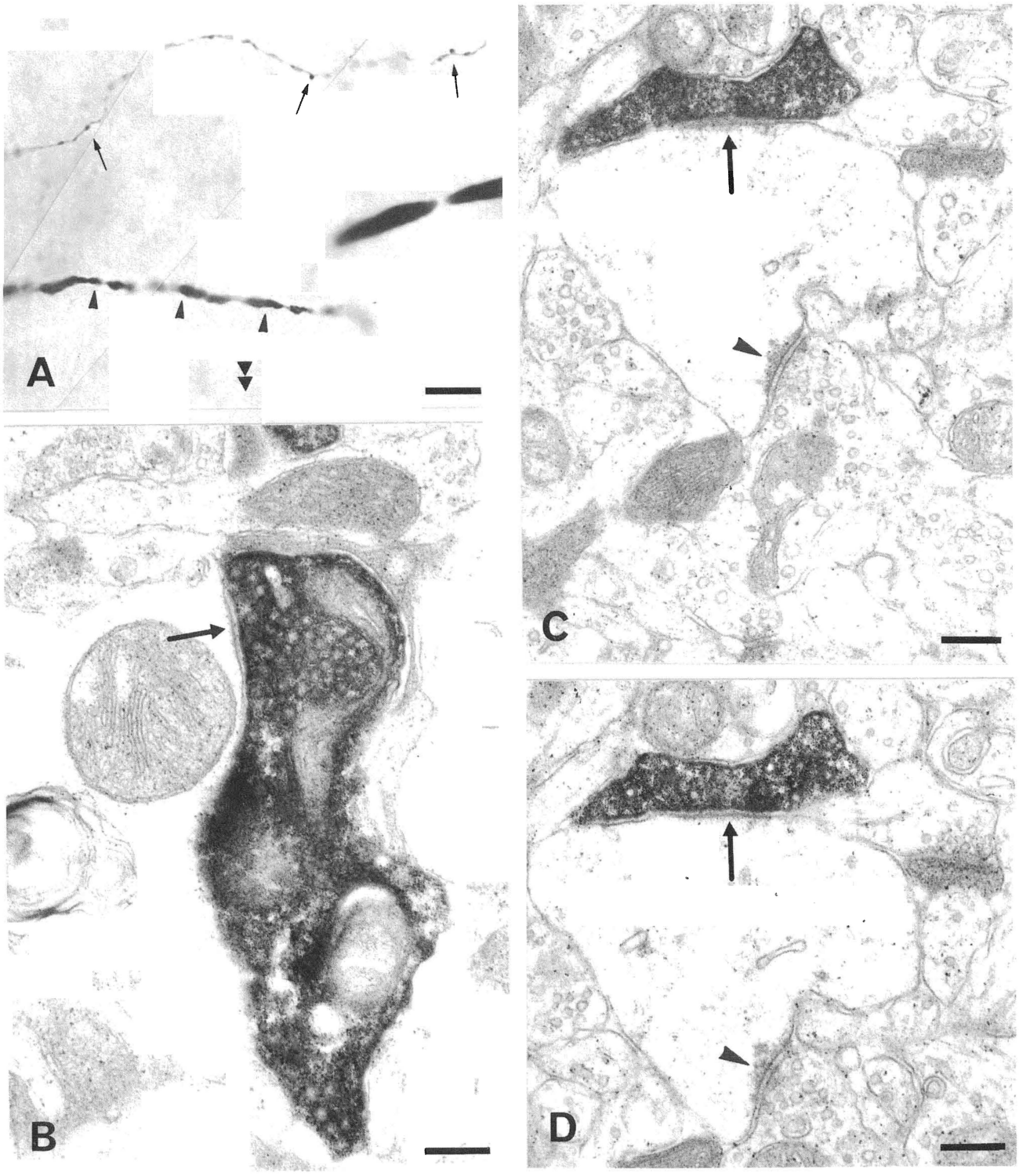

FIG. 6. Light (A) and electron micrographs (B – D) of a MOPP cell. (A) Characteristic sparsely varicose (arrows) axon collateral and a dendrite (arrowheads). Double arrowhead points towards the granule cell layer. (B) Type 2 symmetrical synapse (arrow) made by a biocytin-filled bouton with a non-filled dendrite. (C, D) Serial sections of a biocytin-filled axon forming a type 2 synapse (arrow) with a dendrite. Note that the presynaptic profile is very narrow, no bouton-like enlargement is observed. Arrowhead shows a type 1 synapse on the same dendritic profile. Scales: A, 20 μm; B – D, 0.2 μm.

The selective distribution of the dendrites (Han *et al.*, 1993) also predicts that differences in afferent activation of these cells are very likely to occur. Thus, the synaptic organization of each cell type presents constraints and specificity for their role in the circuit. Grouping all large GABAergic cells which have somata and axons in and near the granule cell layer into a category as basket cells disregards their selectivity and multiplicity in local circuits, and is unlikely to lead to meaningful explanations. It is therefore suggested that the term *basket cell* is used

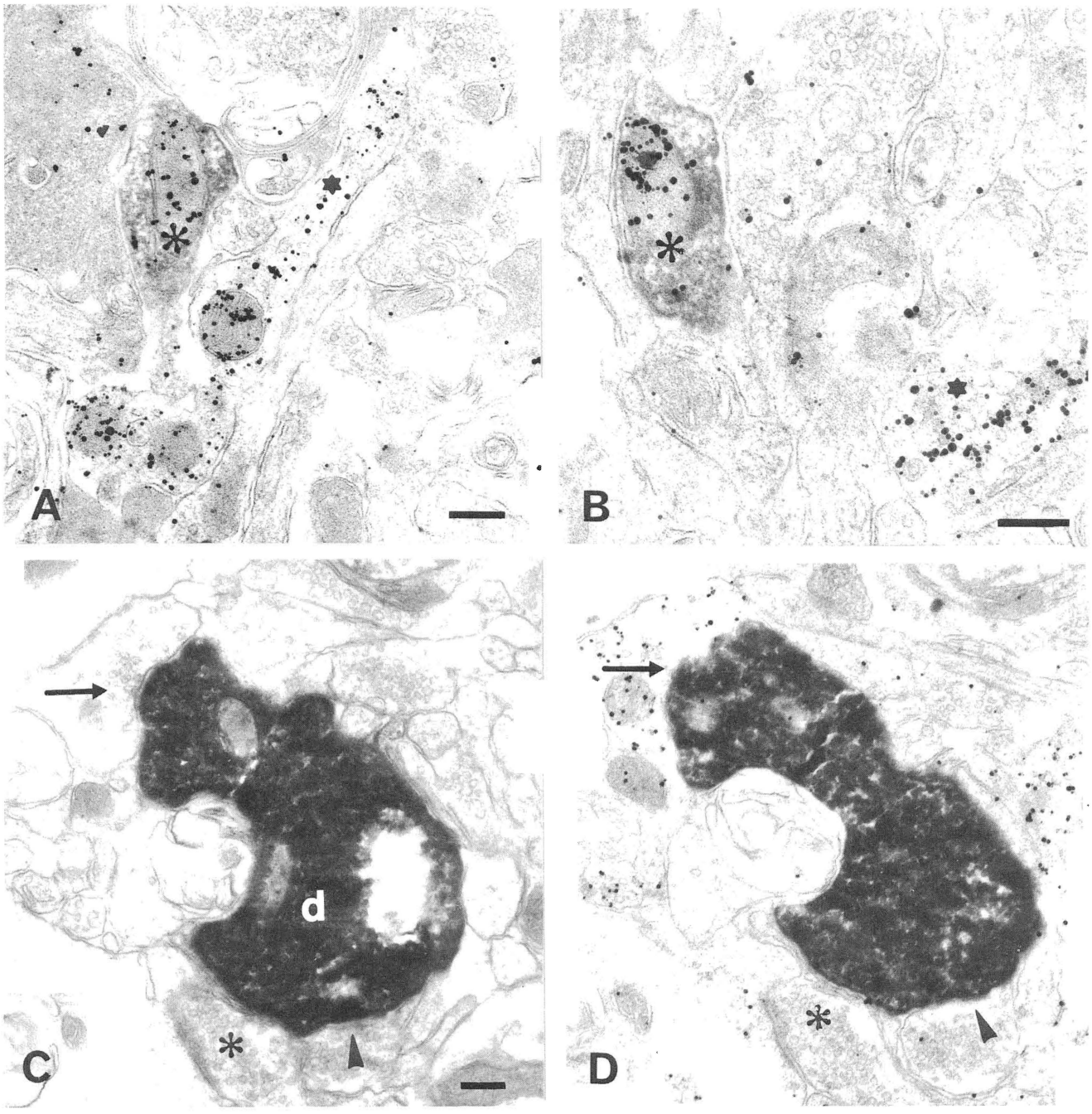

FIG. 7. GABA immunoreactivity of the MOPP cell and its synaptic input. Sections shown in A, B and D were immunoreacted for GABA by silver-intensified postembedding gold reaction. (A, B) Axonal varicosities (asterisks) filled with biocytin are densely labelled by metal particles. Stars indicate GABA-immunopositive non-filled axons. (C, D) Serial sections showing a non-spiny dendrite (d) of the MOPP cell. It receives synapses from a GABA-positive bouton (arrow) and from a GABA-negative bouton (arrowhead). Asterisk marks another bouton forming a type 1 synapse on a non-filled dendrite. Scales: A–D, 0.2 μm; C and D are at the same magnification.

in a more restricted sense than previously, and should be applied only to cells which can be shown to distribute their efferent synapses on the somata and proximal dendrites of granule cells.

Our results provide morphological evidence for the spatial and postsynaptic target selective termination of different subsets of local circuit neurons. The question arises whether the targets of the five types of neuron are in fact granule cells. It is very likely that the majority of the postsynaptic targets of the dentate basket cell are granule cell somata, as the morphological characteristics of the granule cell body are distinguishable from those of non-principal cells (Seress and Ribak 1984; Leranth *et al.*, 1990; Soriano *et al.*, 1990). Similarly, most if not all axon initial segments can only originate from granule cells in the cell body layer. It is more difficult to define the origin of the dendrites postsynaptic to the three cell types terminating in the molecular layer, because hilar cells (Scharfman, 1991), as well as some cells from the CA3 area of the hippocampus (Amaral, 1978), also send dendrites into the dentate molecular layer. The dendrites of non-principal cells can be excluded as major postsynaptic targets of HICAP, HIPP and MOPP cells,

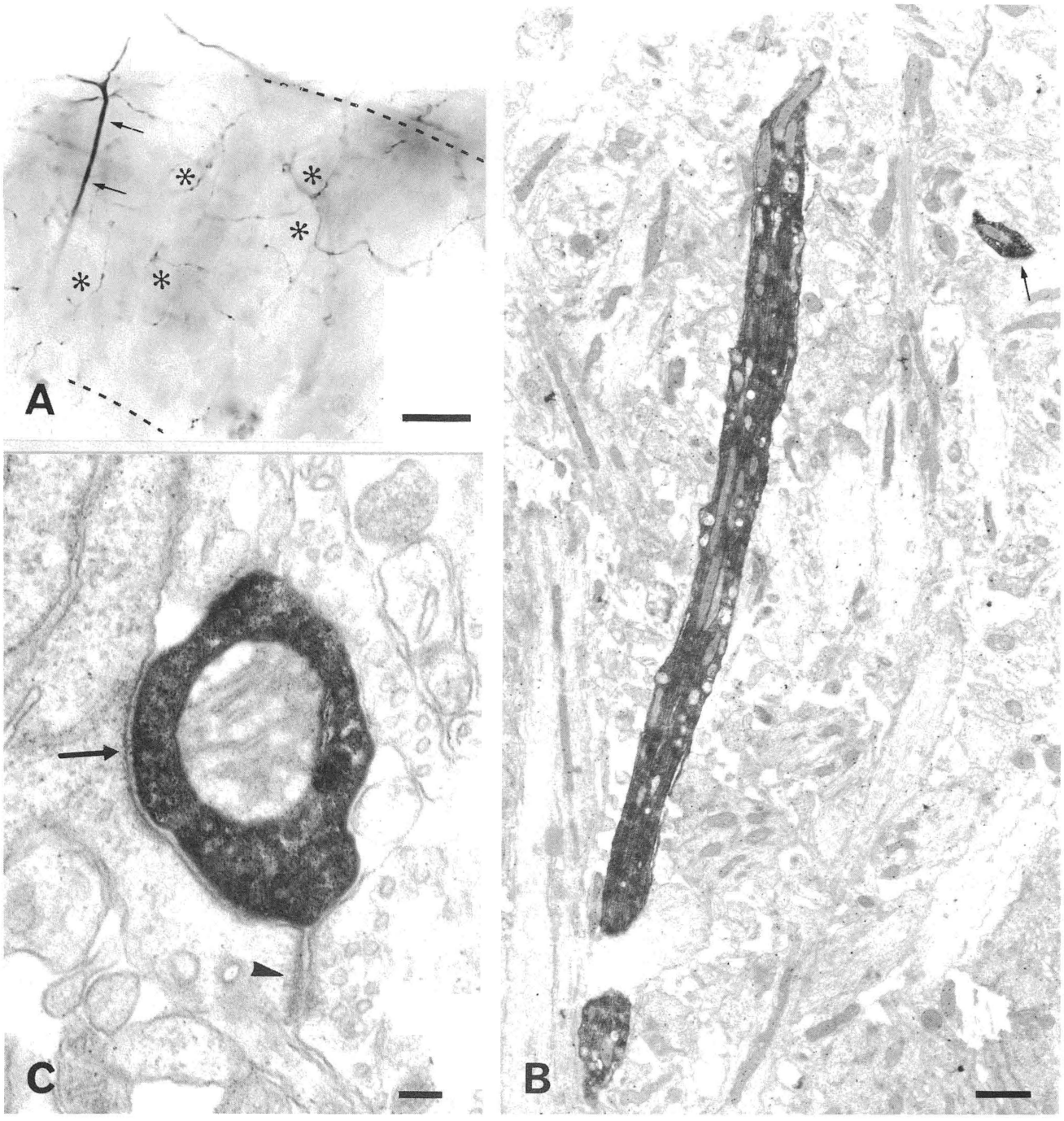

FIG. 8. Light (A) and electron micrographs (B, C) of a basket cell. (A) The main axon (arrows) and the characteristic waving axon collaterals among the granule cells (asterisks). Broken lines follow the border of the granule cell layer. (B) The unmyelinated main axon ascends to the inner molecular layer. A biocytin-filled synaptic bouton (arrow) is also seen. (C) A type 2 synapse (arrow) is established by the biocytin-filled bouton at the neck of a somatic spine originating from a granule cell. Arrowhead marks a type 1 synapse on the same spine. Scales: A, 20 μm; B, 1 μm; C, 0.1 μm.

because GABAergic cells generally have few spines in the hippocampal formation and the majority of postsynaptic dendrites emitted spines. Furthermore, none of the tested dendrites postsynaptic to the identified biocytin-filled boutons was immunopositive for GABA.

The presence of spines on the dendrites was the main morphological criterion used to predict that the dendrite originated from granule cells. This criterion would also include possible dendrites of mossy cells and CA3 pyramidal cells and we cannot exclude that they contribute to the postsynaptic targets in the molecular layer. However, the proportion is probably minute in comparison to the granule cell dendrites; the majority of the postsynaptic targets of HICAP, HIPP and MOPP cells were therefore probably granule cell dendrites. Some postsynaptic dendritic shafts could not be shown to emit spines, but this is probably due to the small diameter of the spine necks, which only appear at favourable section planes even in optimally fixed tissue. The long survival time of some of the slices and the immersion fixation method substantially

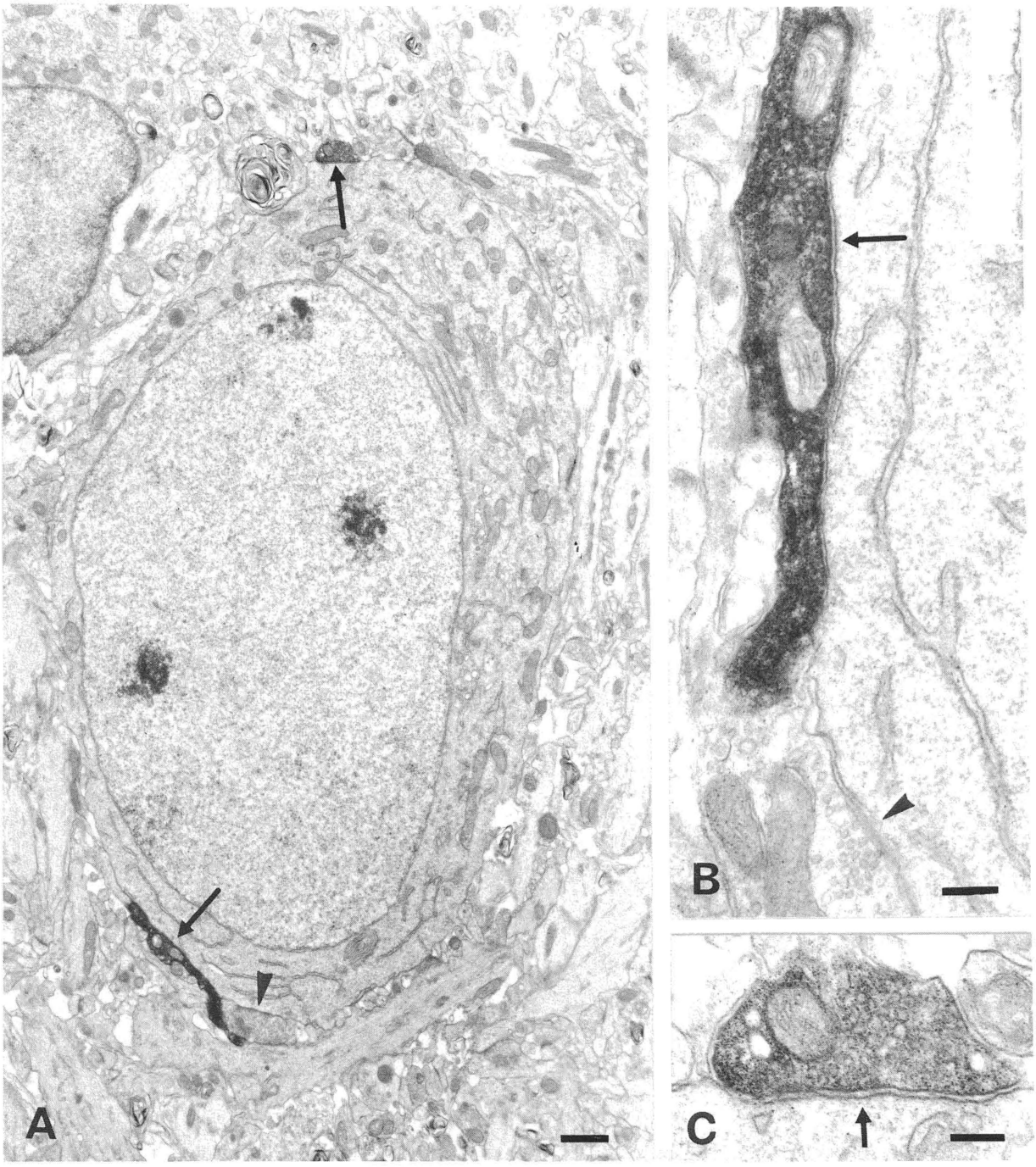

FIG. 9. Electron micrographs of the same dentate basket cell shown in Figure 8. (A) A granule cell is in synaptic contact (arrows) with two biocytin-labelled boutons (arrows) and a non-labelled bouton (arrowhead). (B, C) Higher magnification micrographs showing type 2 synaptic contacts (arrows) with the granule cell soma. Arrowhead in B (serial section to A) shows a similar synapse made by a non-labelled bouton. Scales: A, 1 μm; B and C, 0.2 μm.

reduced the chances of detecting spines connected to dendritic shafts in the present material. In conclusion, although it cannot be excluded that there were some genuine aspinous dendrites among the postsynaptic targets, the majority of the dendritic and spine targets belonged to granule cells.

The above conclusion is further supported by the presence of dendritic spines among the targets of the HICAP and HIPP cells. Their proportion (29.5% and 25% respectively) is comparable to the 26% of dendritic spines amongst the targets of all GABAergic synapses in the cat and monkey visual cortex (Beaulieu and Somogyi, 1990; Beaulieu *et al.*, 1992). Furthermore, the present results identify at least two different types of GABAergic cells as the source of GABA to dendritic spines described recently in the rat dentate gyrus (Fifkova *et al.*, 1992). The type 2 synapses of HICAP and HIPP cells on dendritic spines were seen in association with type 1 synapses on the same spines. The possible physiological significance of this arrangement will be discussed below.

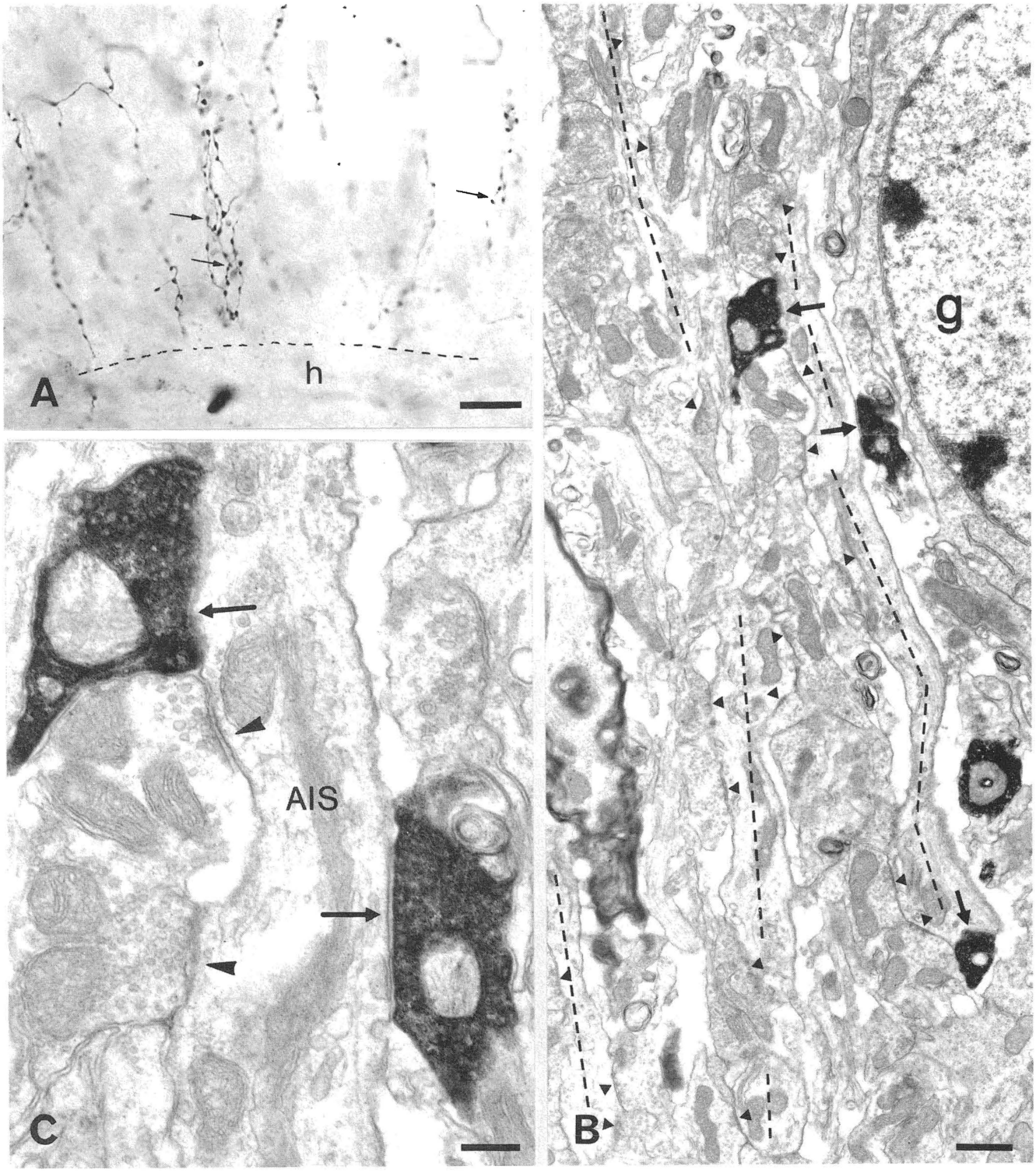

FIG. 10. Light (A) and electron micrographs (B, C) of a dentate axo-axonic cell. (A) Rows of biocytin-filled boutons (arrows) following the course of the axon initial segments of granule cells. Broken line indicates the border of hilus (h) and granule cell layer. (B) Electron micrograph of a longitudinally cut bundle of axon initial segments (broken lines) in the granule cell (g) layer. Three biocytin-filled (arrows) and several times more non-filled (triangles) boutons establish synaptic contacts with the initial segments. (C) The two uppermost biocytin-filled boutons in B are shown at higher magnification. Arrows mark type 2 synapses given by axo-axonic cell boutons; arrowheads show synapses of non-filled boutons on the same axon initial segment (AIS). Scales: A, 20 μm; B, 0.5 μm; C, 0.2 μm.

Surprisingly, two different types of synapses were made by the same HICAP cell on dendritic spines. It is very unlikely that this finding was a result of dye-coupling (Kawaguchi *et al.*, 1989) of another axon, not belonging to the HICAP cell, and the filled cell, since both types of bouton were GABA-immunopositive, and in one case we also followed the same axon collateral giving both asymmetrical and symmetrical

TABLE 1. Distribution of the postsynaptic targets of five types of identified neuron

Cell type	Postsynaptic targets			
	Dendritic shaft	Dendritic spine	Granule cell soma	Axon initial segment
HICAP cell	24 (70%)	10 (30%) (4[a])	–	–
HIPP cell	19 (75%)	6 (25%)	–	–
MOPP cell	20 (100%)	–	–	–
Dentate basket cell	9 (22%)	–	32 (78%) (6[b])	–
Axo-axonic cell	–	–	–	14 (100%)

[a]Number of type 1 synapses.
[b]Number of synapses on somatic spines.

synapses. Synaptic terminals immunoreactive for GAD have been reported to make asymmetrical synapses with the somata of granule cells (Kosaka *et al.*, 1984). We have found that GABA-positive boutons that form asymmetrical type 1 synapses on dendritic spines also occur in normal optimally fixed hippocampal tissue (K. Halasy and P. Somogyi, unpublished observation), in the same zone in the molecular layer where the terminals of the HICAP cell were found. It is possible that the two types of synapses made by the HICAP cell are coupled to different receptor mechanisms, and the difference in the subsynaptic molecular constituents results in morphological distinction of the two types of synapses.

Convergence and divergence in local synaptic connections

In the rat, the ratio of 'basket cells' to granule cells is estimated to be 1:200 (Seress and Pokorny, 1981), but the basket cells were only identified as non-granule cells and might include other non-principal cell types. It is probable that a basket cell with such an extensive termination field as shown by Han *et al.* (1993) is in synaptic connection with far more than 200 and probably with thousands of granule cells. Thus, the convergence of numerous basket cells on one granule cell must take place. Indeed, we found that in addition to the biocytin-filled basket cell terminals, non-filled boutons giving similar type 2 synapses on the granule cell bodies were usually present. These boutons could belong to other basket cells. Unfortunately, the suboptimal fine structural preservation of the slice containing this cell did not allow the quantitative evaluation of the degree of convergence. In contrast to the 'basket' formation expected from classical accounts (Ramon y Cajal, 1893; Lorente de No, 1934), we found that any individual granule cell receives few, probably < 10 and often only one, synapses from the identified basket cell axon.

As found in previous studies (Kosaka, 1983; Soriano and Frotscher, 1989; Soriano *et al.*, 1990) convergence was evident also on the axon initial segments supplied by the axo-axonic cell. In the CA1 region of the monkey it was calculated that at least three axo-axonic cells converged on one pyramidal cell axon initial segment (Somogyi *et al.*, 1983). The degree of convergence has not been established in any region of the rat hippocampus, but from indirect calculations it has been estimated that 4–10 axo-axonic cells might converge on one initial segment in the rat CA1 region (Li *et al.*, 1992).

The number of neurons postsynaptic to axo-axonic cells can be estimated reasonably accurately due to the special formation of grouped boutons forming 'candlesticks' (Szentagothai and Arbib, 1974), each corresponding to one postsynaptic axon initial segment innervated by the chandelier axon. Thus, previous estimates range from 323 in the CA1 region of the cat, based on partial reconstruction (Somogyi *et al.*, 1985), to 1214 innervated pyramidal cells in the rat (Li *et al.*, 1992) obtained from an intracellularly filled cell having a completely reconstructed axonal arborization. The degree of divergence is probably much greater for the hilar chandelier cell studied here, and may include many thousands of granule cells. However, because the chandelier axon fascicles in the granule cell layer follow bundles of axon initial segments, the real number of innervated individual granule cells could not be estimated.

The dendrites postsynaptic to the terminals of the HICAP, HIPP and MOPP cells were not found to receive other GABA-positive synaptic boutons, but in all cases only small segments were studied. The surrounding tissue contained other GABA-positive synaptic terminals not belonging to the identified cell and indicating that at least the same molecular layer space is supplied by converging GABAergic input which may originate from different cell types and/or from other individual members of the same class.

GABA in synaptic terminals

Axo-axonic and basket cells are thought to exert their influence through GABA as a transmitter. This assumption is based on their immunoreactivity for GABA (Somogyi *et al.*, 1985; Soriano and Frotscher, 1989) or for GAD (Ribak *et al.*, 1978). The HICAP and MOPP cells also have been shown to be immunoreactive for GABA in our experiments.

No information has been obtained for the possible transmitter of the HIPP cell. It should be noted that the immunoreactivity of this slice for GABA was weaker than the other slices and this may have produced false negative results. However, since HIPP cells probably correspond to somatostatin/neuropeptide Y-immunopositive neurons (Han *et al.*, 1993), it is noteworthy that GABA/somatostatin-immunoreactive neurons gave a weaker GABA immunoreaction than somatostatin-negative, GABA-immunopositive cells (Somogyi *et al.*, 1984). Therefore, it is possible that the normally lower GABA level in the HIPP cell contributed to the lack of GABA immunoreactivity in the slice preparation. In perfusion-fixed material of normal rat dentate gyrus almost all boutons forming type 2 synapses in the outer molecular layer, where the HIPP cell terminates, were GABA-immunopositive (K. Halasy and P. Somogyi, unpublished result). Considering these indirect lines of evidence together with the technical difficulties of immunoreaction in slice tissue, it seems probable that the HIPP cell is a GABA- and somatostatin-containing neuron.

The dendrites of both the HICAP and MOPP cells received synapses from many GABA-immunoreactive boutons in addition to GABA-negative ones. The GABAergic input to GABAergic neurons could originate from the medial septum (Bilkey and Goddard, 1985; Freund and Antal, 1988; Gulyas *et al.*, 1990) or from local cells. Input from either source could provide inhibition to the GABAergic cells, thus resulting in disinhibition of the granule cells. However, it has been suggested that GABAergic neurons in the hilus may provide $GABA_A$ receptor-mediated excitatory input to each other, which would synchronize the firing of GABAergic hilar cells (Michelson and Wong, 1991). The identity of the cell types that might provide this excitatory GABAergic input and any role in physiological processes has not been determined.

Roles of local GABAergic inputs

The differences in the connections of distinct classes of GABAergic cells converging on granule cells suggest that each cell type has a distinct physiological role. The final outcome of a GABA-mediated effect on the principal neurons depends on (i) the location of the synapses on the postsynaptic cell, (ii) the types of GABA receptor mediating the action, and (iii) dynamic interactions with other inputs active at the time of GABA release.

Location of synapses

The dentate basket and axo-axonic cell types have been suggested to be inhibitory (Ribak *et al.*, 1978; Soriano *et al.*, 1990) by analogy with similar cell types in the CA1 region (Kandel *et al.*, 1961; Andersen *et al.*, 1964a, b; Somogyi *et al.*, 1983). In the latter area, the most sensitive site to GABA-mediated inhibition was found to be in the somatic layer (Andersen *et al.*, 1964a, b), where both basket and axo-axonic cells terminate. Exogenously applied GABA evokes a bicuculline-sensitive hyperpolarization in this layer (Newberry and Nicoll, 1985). Similarly in the dentate gyrus the maximum of the orthodromically evoked IPSP was found near the granule cell layer (Andersen *et al.*, 1966). However, it remains a puzzle why two distinct sources of GABA evolved for the soma and axon initial segment, and the direct synaptic effect of these morphologically distinct cell types remains to be established.

Assuming that both basket and axo-axonic cells are inhibitory, the location of their synapses suggests that they control the final output of granule cells. It has been suggested that axo-axonic cells might control the threshold for action potential generation of large populations of principal cells (Somogyi *et al.*, 1982, 1983; Douglas and Martin, 1990), and simultaneously they might synchronize them (Somogyi *et al.*, 1983). A synchronizing role would be particularly appropriate in the dentate gyrus, where the granule cells fire preferentially on the positive phase of the rhythmic field potential during theta activity (Andersen, 1980; Buzsaki *et al.*, 1983; Munoz *et al.*, 1990). The activation threshold to perforant path stimulation of some presumed inhibitory cells in the hilus is lower than that of granule cells (Scharfman, 1991), supporting a role for one or both of these inhibitory neurons in setting the threshold of granule cells to afferent activation in a feed-forward manner (Buzsaki, 1984).

The GABAergic synapses in the dendritic tree, identified here as originating from specific types of dentate cells, probably have a more local effect on the responses of the specific innervated dendritic segments than on the output of the cell. However, granule cells are electrotonically relatively compact (average electrotonic length of equivalent cable $L = 0.49$; Staley *et al.*, 1992); thus theoretically the conductance changes in the dendrites may also affect the responsiveness of the whole cell (Blaxter and Carlen, 1988).

Multiple receptor mechanisms

Granule cells and hippocampal pyramidal cells respond to GABA through both $GABA_A$ and $GABA_B$ postsynaptic receptors (Thalmann and Ayala, 1982; Misgeld *et al.*, 1986, 1992; Rausche *et al.*, 1989; Muller and Misgeld, 1990; 1991; Mott and Lewis, 1991; but see also Steffensen and Henriksen, 1991). Some of the GABA-mediated inhibition is provided by hilar cells (Muller and Misgeld, 1990; 1991; Scharfman *et al.*, 1990; Misgeld *et al.*, 1992), but these have not been adequately identified anatomically to allow correlation with the four cell types characterized here in the hilus. The $GABA_A$ and $GABA_B$ receptor-mediated responses can be evoked independently in granule cells (Muller and Misgeld, 1990), indicating that the synapses of some local circuit cells may only use one of the postsynaptic receptor subtypes. The subcellular distribution of the different GABA receptors on the granule cell surface is not known. In the CA1 region only $GABA_A$ receptors are thought to be present on and near to the soma (Newberry and Nicoll, 1985), but both receptor mechanisms operate in the dendritic region of pyramidal cells (Newberry and Nicoll, 1985). Nevertheless, pure $GABA_B$ receptor-mediated postsynaptic inhibition can be evoked (Segal, 1990) in pyramidal cells, and IPSPs similar to $GABA_B$ receptor-mediated postsynaptic responses have been shown to be produced by GABAergic cells located and terminating in the dendritic field of the CA1 region (Lacaille and Schwartzkroin, 1988). By analogy it is not unreasonable to assume that the MOPP cell located and terminating in the molecular layer acts only on $GABA_B$ receptors in the dendritic tree of the granule cells.

A further difference in the physiological effect of the different GABAergic cells may arise from the possible selective distribution of different $GABA_A$ receptor subunits (for review see Luddens and Wisden, 1991). Granule cells express at least 11 of the known $GABA_A$ receptor subunits, as shown by *in situ* hybridization (for summary see Wisden *et al.*, 1992). Since only five subunits are thought to constitute the native receptor complex, a large variety of differently composed receptor subtypes may be present on the surface of the cell. Immunoreactivity for the $\alpha 1$ subunit was found both on somata and throughout the dendritic field of granule cells (Houser *et al.*, 1988). Interestingly, a change in the density of receptor immunoreactivity can be seen in the molecular layer corresponding to the termination fields of the HICAP versus the HIPP and MOPP cells (P. Somogyi, unpublished result), indicating possible differences in subunit composition of $GABA_A$ receptors at the different synapses. The same synapses may also involve several distinct $GABA_A$ receptor channel complexes. Granule cell somatic membrane patches show at least two single-channel conductances (14 and 23 pS) in response to GABA (Edwards *et al.*, 1990), and these may represent distinct channels. Recent results obtained from the cerebellum also indicate that at the Golgi cell-to-granule cell GABAergic synapse more than one channel complex type is present (Baude *et al.*, 1992). Further definition of $GABA_A$ receptor subtypes is necessary at the specific synapses supplied by the different local neurons.

Interaction of GABAergic synapses with other inputs

The location of the HICAP, HIPP and MOPP cell synapses in the dendritic tree suggests that they do not control the overall activity of the cell, but interact selectively with other inputs to the same dendritic segment. This is illustrated most vividly by the GABAergic synapses provided to spines, where they converge with another single synapse that exhibits features characteristic for glutamatergic boutons (Grandes and Streit, 1991). The association with excitatory afferents is also evident from the precise segregation of the GABAergic axonal fields according to the two major spatially separated glutamatergic inputs to the dendritic tree. Three mechanisms may be served by the dendrite-selective GABAergic terminations:

(1) *The control of glutamate release through presynaptic $GABA_B$ receptors on the glutamatergic terminals*, suggested to be present for example in the CA3 region of the hippocampus (Thompson and Gahwiler, 1992). However, the GABAergic terminals in the molecular layer are not oriented towards the glutamatergic terminals, but instead form classical synaptic junctions with the granule cell dendrites; therefore postsynaptic explanations should be also considered.

(2) *The control of NMDA receptor activation on specific dendritic segments* may critically depend on the membrane potential level. In the CA1 region (Collingridge *et al.*, 1988; Davies *et al.*, 1990, 1991) as well as in dentate granule cells (Mott and Lewis, 1991; Staley and Mody, 1992) NMDA receptor activation has been shown to be attenuated by $GABA_A$ receptor-mediated inhibition. The entorhinal input acts through both NMDA and non-NMDA receptor mechanisms (Errington *et al.*, 1987; Dahl *et al.*, 1990; Lambert and Jones, 1990; Staley and Mody, 1992). Furthermore, it was shown that GABAergic mechanisms govern the induction of LTP in the dentate gyrus (Tomasulo *et al.*, 1991), and a reduction in GABAergic inhibition is necessary to induce LTP through the NMDA receptor-mediated mechanism (Davies *et al.*, 1990, 1991; Mott and Lewis, 1991). The great degree of overlap in the time course of NMDA and $GABA_A$ receptor-mediated currents results in a substantial shunting of the NMDA receptor-mediated current (Staley and Mody, 1992). The GABAergic circuits revealed here as terminating in

the dendritic tree provide a highly localized mechanism for regulating both the membrane potential and conductance, as well as the probability of LTP in specific dendritic subdivisions. The advantage of pairing different GABAergic cells with the distinct glutamatergic inputs is that it becomes possible to regulate the gain of the inputs and the probability of LTP independently. In turn, these different types of neurons may be specifically influenced by subcortical afferents transmitting information related to the behavioural context and exerting state control over the system. Local circuit GABAergic neurons are heavily and selectively innervated by subcortical afferents (Freund and Antal, 1988; Freund *et al.*, 1990; Gulyas *et al.*, 1991; Halasy *et al.*, 1992), providing an appropriate machinery for such a role.

(3) *The downward rescaling of EPSPs by pathway-specific GABA-mediated events could extend the dynamic range of EPSPs* whatever the glutamate receptor mechanism is for the EPSP. Depending on the threshold and time course for the activation of the GABAergic neurons relative to granule cells, there may be a temporal overlap between the GABA receptor-mediated and both the non-NMDA and the NMDA receptor-mediated events as found *in vitro* (Staley and Mody, 1992). Dendritic segment specific GABAergic mechanisms could then reduce proportionally EPSPs produced in specific parts of the dendritic tree, making EPSPs, which would have been suprathreshold in the absence of GABA receptor activation, subthreshold and available for spatial and temporal summation. This may be a general role for GABAergic terminals which are selectively colocalized in the dendritic tree with glutamatergic terminals in many parts of the central nervous system.

The mechanisms discussed above are not mutually exclusive. The recognition of the structural arrangements now opens them up for physiological exploration.

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Abbreviations

DAB	3,3′-diaminobenzidine tetrahydrochloride
EPSP	excitatory postsynaptic potential
GABA	γ-aminobutyric acid
GAD	glutamate decarboxylase
HICAP	hilar cell with axon in the commissural association pathway termination field
HIPP	hilar cell having an axon associated with the perforant path projection
IPSP	inhibitory postsynaptic potential
LTP	long-term potentiation
MOPP	molecular layer neuron having an axon associated with perforant path input
NMDA	*N*-methyl-D-aspartate

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